PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

MARGOLIS, David, et al.

Divisional of Appln. No.: 09/210,578 Group Art Unit: Not Yet Assigned

Confirmation No.: Not Yet Assigned Examiner: Not Yet Assigned

Filed:

For: INTEGRATIVE PROTEIN-DNA COCHLEATE FORMULATIONS AND METHODS FOR TRANSFORMING CELLS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE TITLE:

Page one, please delete lines 1-2 and insert therefore:

INTEGRATIVE PROTEIN-DNA COCHLEATE FORMULATIONS AND METHODS FOR TRANSFORMING CELLS

IN THE SPECIFICATION:

Amend the specification by inserting before the first line the sentence:

--This is a divisional of Application No. 09/210,578 (Confirmation No. Not Assigned) filed December 14, 1998, the disclosure of which is incorporated herein by reference.--

Page 11, paragraph encompassing lines 7-12:

The individual lipid elements of the layered lipid bilayer of the cochleate precipitates can be any of the many known lipid structures having a negatively charged polar head group. Preferably the majority of the lipid elements of the lipid bilayer contain a negatively charged phospholipid headgroup. Upon contact with a lipid bilayer of a target cell, the layered lipid bilayer is capable of delivering one or more of the therapeutic nucleotide sequences and one or more AAV proteins to the interior of the target cell.

Page 18, paragraph encompassing lines 13-16:

Macroscopically, the final formulations consisted of heavy white suspensions. Phase contrast, light microscopic observation (1000x) indicated heavy suspensions of refractile granular crystals,in both free and aggregate form. Cochleate structure of the crystals was confirmed by addition of EDTA, which caused conversion of the cochleate crystals to liposomes.

Page 18, paragraph encompassing lines 18-23:

Conditions to promote formation of DNA-binding protein complexes may vary but can be determined experimentally. Conditions used were TES buffer (100 mM NaCl, 2 mM TES, 2 mM histidine, pH 7.4) at approximately 2 times the volume of protein in the buffer it was purified in (HEPES buffered, pH 7.5, 150 mM KCl, 1 mM $MgCl_2$, 0.1 mM EDTA, and 10 mM maltose) using a ratio of DNA to lipid of 1.0:10.0 by weight. A probable range of useful ratios for formulations would be from 1:1 to 1:100 by weight.

Page 21, in the Table, first line as follows:

Neonatal cord blood $CD34^+$ cells in 1 mg/ml G418	
Cochleate Type	Colonies/30 fields*
No Cochleates	20
CWRSVN Cochleates alone	23
CWRSVN Cochleates/Rep 68	26
CWRSVN Cochleates/Rep 78	27
CWRSVN Cochleates/Rep 68 and Rep 78	38

*mean of duplicate plates

Page 23, in the Table, last line as follows:

Neonatal cord blood CD34+ cells in 1.5 mg/ml G418		
	Colonies/30 Fields*	
Cochleate Type	Range	Mean
No cochleate	0	0
No cochleates/no G418	60-65	62
G1EN cochleate/Rep 68 & 78	0	0
CWRSVN cochleate alone	0-3	1.5 (sd 1.1)
G1EN retroviral vector**	1-2	1.4 (sd 0.2)
G1EN retroviral vector ***	16-26	18.3 (sd 5.4)
CWRSVN cochleate/Rep 68 & 78	2-8	5.25 (sd 2.2)

* counted in quadruplicate plates
** standard MMLV retroviral vector expressing neo resistance
*** transduced with cytokines for 3 days (standard retroviral transduction procedure)

IN THE CLAIMS:

Please cancel claims 1-88 without prejudice or disclaimer.

Please add the following new claims:

89. A composition comprising:

a) a vector delivery structure comprising:

1) a cochleate comprising a lipid bilayer element and cations;

2) one or more proteins that facilitate the integration of one or more nucleotide sequences, that express a molecule, into the genome of a host cell; and

3) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences; and

b) a pharmaceutically acceptable carrier.

90. The composition of claim 89, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

91. The composition of claim 89, wherein the vector delivery structure comprises a polynucleotide that expresses one or more proteins that facilitate integration.

92. The composition of claim 89, wherein the cations are divalent cations.

93. The composition of claim 89, wherein the cations are calcium.

94. The composition of claim 89, wherein the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of a host cell are one or more binding proteins that have a DNA binding motif.

95. The composition of claim 94, wherein the one or more binding proteins are from adeno-associated virus type II.

96. The composition of claim 94, wherein the one or more binding proteins are at

least one adeno-associated virus protein selected from the group consisting of Rep 68 and Rep 78.

97. The composition of claim 94, wherein the one or more binding proteins comprise Rep 68 and Rep 78.

98. The composition of claim 89, 96 or 97, wherein the one or more DNA sequences recognized and bound by the one or more proteins are the inverted terminal repeat regions of adeno-associated virus.

99. The composition of claim 98, wherein the one or more oligonucleotides or polynucleotides comprise a length of DNA that is flanked at each end by at least one of the inverted terminal repeat regions.

100. The composition of claim 94, wherein at least one of the one or more binding proteins is an integrase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said integrase.

101. The composition of claim 100, wherein at least one of the one or more binding proteins is an integrase that is not Rep 68 or Rep 78.

102. The composition of claim 94, wherein at least one of the one or more binding proteins is a helicase and at least one of the one or more sequences recognized and bound by the one or more DNA binding proteins is a substrate for said helicase.

103. The composition of claim 94, wherein at least one of the one or more binding proteins is a DNA excision enzyme and at least one of the one or more DNA sequences

recognized and bound by the one or more binding proteins is a substrate for said DNA excision enzyme.

104. The composition of claim 94, wherein at least one of the one or more binding proteins is an isomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said isomerase.

105. The composition of claim 94, wherein at least one of the one or more binding proteins is a telomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said telomerase.

106. The composition of claim 94, wherein at least one of the one or more binding proteins is a DNA repair enzyme and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said DNA repair enzyme.

107. The composition of claim 89, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of a host cell is a protein that has gene regulatory activity and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said protein that has gene regulatory activity.

108. The composition of claim 89, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of a host cell is a protein that facilitates transport to or uptake by the nucleus of the host cell.

109. The composition of claim 89, wherein the host cell is a human cell.

110. The composition of claim 89, wherein the host cell is a pluripotent stem cell.

111. A composition comprising:

a) a vector delivery structure for delivering to the interior of a host cell one or more therapeutic nucleotide sequences, that express a molecule, and one or more proteins that bind to DNA for facilitating the integration of the one or more nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

1) a cochleate comprising a lipid bilayer element wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

2) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein and a Rep 78 protein; and

3) a polynucleotide comprising one or more inverted terminal repeat regions of adeno-associated virus type II and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequence; and

b) a pharmaceutically acceptable carrier.

112. The composition of claim 111, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

113. The composition of claim 111, wherein the one or more oligonucleotides or

polynucleotides comprise a length of DNA that is flanked at each end by at least one inverted terminal repeat region.

114. The composition of claim 111, 112 or 113, wherein the vector delivery structure comprises a polynucleotide that expresses one or more proteins that facilitate integration.

115. The composition of claim 111, wherein the host cell is a human cell.

116. The composition of claim 111, wherein the host cell is a pluripotent stem cell.

117. A method for transforming a host cell in vitro with one or more nucleotide sequences, that express a molecule, the method comprising transfecting a host cell in vitro with a vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element and cations;

b) one or more proteins that facilitate the integration of said one or more nucleotide sequences into the genome of said host cell; and

c) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences.

118. The method of claim 117, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

119. The method of Claim 117, wherein the vector delivery structure comprises a polynucleotide that expresses one or more proteins that facilitate integration.

120. The method of claim 117, wherein the cations are divalent cations.

121. The method of claim 117, wherein the cations are calcium.

122. The method of claim 117, wherein the one or more proteins that facilitate the integration of one or more nucleotide sequences into the genome of a host cell are one or more binding proteins that have a DNA binding motif.

123. The method of claim 122, wherein the one or more binding proteins are from adeno-associated virus type II.

124. The method of claim 122, wherein the one or more binding proteins are at least one adeno-associated virus protein selected from the group consisting of Rep 68 and Rep 78.

125. The method of claim 122, wherein the one or more binding proteins comprise both Rep 68 and Rep 78.

126. The method of claim 117, 124 or 125, wherein the one or more DNA sequences recognized and bound by the one or more binding proteins are the inverted terminal repeat regions of adeno-associated virus.

127. The method of claim 126, wherein the one or more oligonucleotides or polynucleotides comprise a length of DNA that is flanked at each end by at least one of the inverted terminal repeat regions.

128. The method of claim 122, wherein at least one of the one or more binding proteins is an integrase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said integrase.

129. The method of claim 128, wherein at least one of the one or more binding proteins

is an integrase that is not Rep 68 or Rep 78.

130. The method of claim 122, wherein at least one of the one or more binding proteins is a helicase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said helicase.

131. The method of claim 122, wherein at least one of the one or more binding proteins is a DNA excision enzyme and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said DNA excision enzyme.

132. The method of claim 122, wherein at least one of the one or more binding proteins is an isomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said isomerase.

133. The method of claim 122, wherein at least one of the one or more binding proteins is a telomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said telomerase.

134. The method of claim 122, wherein at least one of the one or more binding proteins is a DNA repair enzyme and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said DNA repair enzyme.

135. The method of claim 117, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of the host cell is a protein that has gene regulatory activity and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said protein that has gene regulatory activity.

136. The method of claim 117, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of the host cell is a protein that facilitates transport to or uptake by the nucleus of the host cell.

137. The method of claim 117, wherein the host cell is a human cell.

138. The method of claim 117, wherein the host cell is a pluripotent stem cell.

139. The method of claim 117 or 138, performed in the absence of cytokine stimulation.

140. The method of claims 117, wherein the in vitro transfected host cells are returned to the same animal or placed in another animal.

141. A method for transforming a host cell in vitro with one or more nucleotide sequences, that express a molecule, the method comprising transfecting said host cell in vitro with a vector delivery structure for delivering to the interior of the host cell said one or more nucleotide sequences and one or more binding proteins for facilitating the integration of the one or more nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element, wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

b) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein or a Rep 78 protein; and

c) a polynucleotide comprising one or more inverted terminal repeat regions

of the adeno-associated virus and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences.

142. The method of claim 141, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

143. The method of claim 141, wherein the one or more oligonucleotides or polynucleotides comprise a length of DNA that is flanked at each end by at least one of the inverted terminal repeat regions.

144. The method of claim 141, 142 or 143, wherein the vector delivery structure comprises a polynucleotide that expresses one or more proteins that facilitates integration.

145. The method of claim 141, wherein the target cell is a human cell.

146. The method of claim 141, wherein the target cell is a pluripotent stem cell.

147. The method of claim 141 or 146, performed in the absence of cytokine stimulation.

148. The method of claim 141, wherein the in vitro transfected host cells are returned to the same animal or placed in another animal.

149. A method for ex vivo treatment of a subject in need thereof, comprising:

a) transforming a host cell in vitro with one or more nucleotide sequences, that express a molecule, with a vector delivery structure comprising:

1) a cochleate comprising a lipid bilayer element and cations;

2) one or more proteins that facilitate the integration of said one or more nucleotide sequences into the genome of said host cell; and

3) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences, and

b) administering said in vitro transformed host cell to said subject.

150. The method of claim 149, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

151. The method of claim 149, wherein the structure includes a polynucleotide that expresses one or more proteins that facilitate integration.

152. The method of claim 149, wherein the cations are divalent cations.

153. The method of claim 149, wherein the cations are calcium.

154. The method of claim 149, wherein the one or more proteins that facilitate the integration of said one or more nucleotide sequences into the genome of said host cell is a binding protein that has a DNA binding motif.

155. The method of claim 154, wherein the one or more binding proteins are from adeno-associated virus type II.

156. The method of claim 154, wherein the one or more binding proteins are at least one adeno-associated virus protein selected from the group consisting of Rep 68 and Rep 78.

157. The method of claim 154, wherein the one or more binding proteins comprise both Rep 68 and Rep 78.

158. The method of claim 149, 156 or 157, wherein the one or more DNA sequences recognized and bound by the one or more proteins are the inverted terminal repeat regions of adeno-associated virus.

159. The method of claim 158, wherein the one or more oligonucleotides or polynucleotides comprise a length of DNA that is flanked at each end by at least one of the inverted terminal repeat regions.

160. The method of claim 154, wherein at least one of the one or more binding proteins is an integrase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said integrase.

161. The method of claim 160, wherein at least one of the one or more binding proteins is an integrase that is not Rep 68 or Rep 78.

162. The method of claim 154, wherein at least one of the one or more binding proteins is a helicase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said helicase.

163. The method of claim 154, wherein at least one of the one or more binding proteins is a DNA excision enzyme and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said DNA excision enzyme.

164. The method of claim 154, wherein at least one of the one or more binding proteins is an isomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said isomerase.

165. The method of claim 154, wherein at least one of the one or more binding proteins is a telomerase and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said telomerase.

166. The method of claim 154, wherein at least one of the one or more binding proteins is a DNA repair enzyme and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said DNA repair enzyme.

167. The method of claim 149, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of the host cell is a protein that has gene regulatory activity and at least one of the one or more DNA sequences recognized and bound by the one or more binding proteins is a substrate for said protein that has gene regulatory activity.

168. The method of claim 149, wherein at least one of the one or more proteins that facilitate the integration of the one or more nucleotide sequences into the genome of the host cell is a protein that facilitates transport to or uptake by the nucleus of the host cell.

169. The method of claim 149, wherein the host cell is a human cell.

170. The method of claim 149, wherein the host cell is a pluripotent stem cell.

171. The method of claim 149 or 170, performed in the absence of cytokine stimulation.

172. A method for ex vivo treatment of a subject in need thereof comprising:

a) transforming a host cell in vitro with one or more nucleotide sequences, that express a molecule, comprising a vector delivery structure for delivering to the interior of the host cell said one or more nucleotide sequences and one or more binding proteins for facilitating the integration of the one or more

nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

1) a cochleate comprising a lipid bilayer element, wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

2) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein or a Rep 78 protein; and

3) a polynucleotide comprising one or more inverted terminal repeat regions of the adeno-associated virus and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences, and

b) administering said in vitro transformed host cell to said subject.

173. The method of claim 172, wherein the polynucleotide is selected from the group consisting of a plasmid or nucleic acid construct.

174. The method of claim 172, wherein the one or more oligonucleotides or polynucleotides comprise a length of DNA that is flanked at each end by at least one of the inverted terminal repeat regions.

175. The method of claim or 172, 173 or 174, wherein the structure includes a polynucleotide that expresses one or more proteins that facilitates integration.

176. The method of claim 172, wherein the host cell is a human cell.

177. The method of claim 172, wherein the host cell is a pluripotent stem cell.

178. The method of claim 172 or 177, performed in the absence of cytokine stimulation.

179. A vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element and cations;

b) one or more proteins that facilitate the integration of one or more nucleotide sequences, that express a molecule, into the genome of a host cell; and

c) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can treat defective genetic material in the genome of said host cell and are selected from the group consisting of a normal beta globin gene, a normal gene for adenosine deaminase, a normal p47 phox gene, a normal p67 phox gene, and a gene encoding antisense RNA against a transforming oncogene contributing to leukemia or lymphoma.

180. A vector delivery structure for delivering to the interior of a host cell one or more nucleotide sequences, that express a molecule, and one or more proteins that bind to DNA for the integration of the one or more nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

b) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein or a Rep 78 protein; and

c) a polynucleotide comprising one or more inverted terminal repeat regions of adeno-associated virus type II and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can treat defective genetic material in the genome of said host cell and are selected from the group consisting of a normal beta globin gene, a normal gene for adenosine deaminase, a normal p47 phox gene, a normal p67 phox gene, and a gene encoding antisense RNA against a transforming oncogene contributing to leukemia or lymphoma.

181. The composition of claims 89 or 111, wherein said one or more nucleotide sequences that express a molecule can treat defective genetic material in the genome of said host cell and are selected from the group consisting of a normal beta globin gene, a normal gene for adenosine deaminase, a normal p47 phox gene, a normal p67 phox gene, and a gene encoding antisense RNA against a transforming oncogene contributing to leukemia or lymphoma.

182. A vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element and cations;

b) one or more proteins that facilitate the integration of one or more nucleotide sequences, that express a molecule, into the genome of a host cell; and

c) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can replace, correct, or modulate functions in metabolic pathways in said host cell and are selected from the group consisting of a normal gene for C1 complement protein inhibitor, a normal gene for a clotting factor, and a normal gene for insulin.

183. A vector delivery structure for delivering to the interior of a host cell one or more nucleotide sequences, that express a molecule, and one or more proteins that bind to DNA for the integration of the one or more nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

b) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein or a Rep 78 protein; and

c) a polynucleotide comprising one or more inverted terminal repeat regions of adeno-associated virus type II and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can replace, correct, or modulate functions in metabolic pathways in said host cell and are selected from the group

consisting of a normal gene for C1 complement protein inhibitor, a normal gene for a clotting factor, and a normal gene for insulin.

184. The composition of claims 89 or 111, wherein said one or more nucleotide sequences that express a molecule can replace, correct, or modulate functions in metabolic pathways in said host cell and are selected from the group consisting of a normal gene for C1 complement protein inhibitor, a normal gene for a clotting factor, and a normal gene for insulin.

185. A vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element and cations;

b) one or more proteins that facilitate the integration of one or more nucleotide sequences, that express a molecule, into the genome of a host cell; and

c) a polynucleotide comprising one or more DNA sequences recognized and bound by the one or more proteins and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can treat cancer in said host cell and are selected from the group consisting of p53, rb (retinoblastoma gene product), ras, myc, fas ligand, and surface receptors.

186. A vector delivery structure for delivering to the interior of a host cell one or more nucleotide sequences, that express a molecule, and one or more proteins that bind to DNA for the

integration of the one or more nucleotide sequences into the genome of the host cell, the vector delivery structure comprising:

a) a cochleate comprising a lipid bilayer element wherein the layers of the lipid bilayer element are bound together by a divalent calcium cation;

b) a DNA binding protein of adeno-associated virus type II comprising a Rep 68 protein or a Rep 78 protein; and

c) a polynucleotide comprising one or more inverted terminal repeat regions of adeno-associated virus type II and one or more oligonucleotides or polynucleotides, each containing said one or more nucleotide sequences;

wherein said one or more nucleotide sequences that express a molecule can treat cancer in said host cell and are selected from the group consisting of p53, rb (retinoblastoma gene product), ras, myc, fas ligand, and surface receptors.

187. The composition of claims 89 or 111, wherein said one or more nucleotide sequences that express a molecule can treat cancer in said host cell and are selected from the group consisting of p53, retinoblastoma, ras, myc fas ligand, and surface receptors.

188. The method of claims 117, 141, 149 or 172, wherein said one or more nucleotide sequences that express a molecule can treat defective genetic material in the genome of said host cell and are selected from the group consisting of a normal beta globin gene, a normal gene for adenosine deaminase, a normal p47 phox gene, a normal p67 phox gene, and a gene encoding antisense RNA against a transforming oncogene contributing to leukemia or lymphoma.

189. The method of claims 117, 141, 149 or 172, wherein said one or more nucleotide sequences that express a molecule can replace, correct, or modulate functions in metabolic pathways in said host cell and are selected from the group consisting of a normal gene for C1 complement protein inhibitor, a normal gene for a clotting factor, and a normal gene for insulin.

190. The method of claims 117, 141, 149 or 172, wherein said one or more nucleotide sequences that express a molecule can treat cancer in said host cell and are selected from the group consisting of p53, rb (retinoblastoma gene product), ras, myc, fas ligand, and surface receptors.

REMARKS

The Amendments to the specification correct obvious typographical and clerical errors.

New claims 90-148 are supported by original claims 29-88. New claims 149-178 are supported by the original method claims, but recite that treatment occurs *ex vivo*. New claims 179-190 are supported by Examples 4, 5 and 6 at pages 25-26 of the specification.

Accordingly, no new matter is added. Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,

Susan J. Mack

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Date: -September 21, 2001

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

Page 1, please delete lines 1-2 and insert therefore:

INTEGRATIVE PROTEIN-~~DAN-~~DNA C~~H~~OCHLEATE FORMULATIONS AND METHODS FOR TRANSFORMING CELLS

IN THE SPECIFICATION:

The specification is changed as follows:

Page 11, paragraph encompassing lines 7-12:

The individual lipid elements of the layered lipid bilayer of the cochleates precipitates can be any of the many known lipid structures having a negatively charged polar head group. Preferably the majority of the lipid elements of the lipid bilayer contain a negatively charged phospholipid headgroup. Upon contact with a lipid bilayer of a target cell, the layered lipid bilayer is capable of delivering one or more of the therapeutic nucleotide sequences and one or more AAV proteins to the interior of the target cell.

Page 18, paragraph encompassing lines 13-16:

Macroscopically, the final formulations consisted of heavy white suspensions. Phase contrast, light microscopic observation (1000x) indicated heavy suspensions of refractile granular crystals,~~ free and in aggregates~~in both free and aggregate form. Cochleate structure of the crystals was confirmed by addition of EDTA, which caused conversion of the cochleate crystals to liposomes.

Page 18, paragraph encompassing lines 18-23:

Conditions to promote formation of DNA-binding protein complexes may vary but can be determined experimentally. Conditions used were TES buffer (100 mM NaCl, 2 ~~Mm~~ mM TES, 2 mM histidine, pH 7.4) at approximately 2 times the volume of protein in the buffer it was purified in (HEPES buffered, pH 7.5, 150 mM KCl, 1 mM $MgCl_2$, 0.1 mM EDTA, and 10 mM maltose) using a ratio of DNA to lipid of 1.0:10.0 by weight. A probable range of useful ratios for formulations would be from 1:1 to 1:100 by weight.

Page 21, in the Table, first line as follows:

Neonatal ~~blood cord~~cord blood $CD34^+$ cells in 1 mg/ml G418	
Cochleate Type	Colonies/30 fields*
No Cochleates	20
CWRSVN Cochleates alone	23
CWRSVN Cochleates/Rep 68	26
CWRSVN Cochleates/Rep 78	27
CWRSVN Cochleates/Rep 68 and Rep 78	38

*mean of duplicate plates

Page 23, in the Table, last line as follows:

Neonatal cord blood CD34+ cells in 1.5 mg/ml G418		
	Colonies/30 Fields*	
Cochleate Type	Range	Mean
No cochleate	0	0
No cochleates/no G418	60-65	62
G1EN cochleate/Rep 68 & 78	0	0
CWRSVN cochleate alone	0-3	1.5 (sd 1.1)
G1EN retroviral vector**	1-2	1.4 (sd 0.2)
G1EN retroviral vector ***	16-26	18.3 (sd 5.4)
~~SWRSVN~~ CWRSVN cochleate/Rep 68 & 78	2-8	5.25 (sd 2.2)

* counted in quadruplicate plates
** standard MMLV retroviral vector expressing neo resistance
*** transduced with cytokines for 3 days (standard retroviral transduction procedure)

IN THE CLAIMS:

Claims 1-88 are canceled.

Claims 89-139 are new.